RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF DOCETAXEL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:A simple, economic, accurate and precise reverse phase liquid chromatographic method was developed for the determination of Docetaxel in pharmaceutical dosage form. Docetaxel is a clinically well established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian and non-small cell lung cancer. The Chromatographic separation was achieved on Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm and Phosphate buffer (pH-3.6): Acetonitrile (27:73) used as a Mobile phase, isocratic mode with flow rate of the mobile phase kept at 1.0 mL/min. The concentration of sample solution was 10µg/mL. The column temperature was maintained at ambient and the detection wavelength was 235nm. The injection volume was 20µl. The method was validated for accuracy, precision, linearity, LOD, LOQ, robustness. The developed method was found to be linear in range of 0-14µg/ml with correlation coefficient (r2)0.999. The precision was estimated by inter-day and intra-day precision and result were calculated as % RSD with found to be within limits. The limit of detection (LOD) and limit of quantification (LOQ) of Docetaxel was found to be 0.09µg/ml and 0.29µg/ml. The method was validated and found good results of precision, Selectivity. The proposed method was found to be suitable and accurate for the quantitative determination of the assay and related sub- stances of Docetaxel active pharmaceutical ingredient.

Key words: Docetaxel, RP-HPLC, Method development, Method Validation, Accuracy, ICH Guidelines.

I. INTRODUCTION

Docetaxel is a clinically well established anti-mitotic chemotherapy medication[1] used mainly for the treatment of breast, ovarian and non-small cell lung cancer. Docetaxel binds to microtubules reversibly with high affinity and has a maximum stoichiometry of one mole docetaxel per mole tubulin in microtubules.

For the treatment of patients with locally advanced or metastatic breast cancer after failure of prior chemotherapy. Also used as a single agent in the treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of prior platinum-based chemotherapy. It is also used in combination with prednisone, in the treatment of patients with androgen independent (hormone refractory) metastatic prostate cancer. Furthermore, docetaxel[2] has uses in the treatment of gastric adenocarinoma and head and neck cancer.

Docetaxel is a taxoid antineoplastic agent[3]. It promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability[4] results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular[5] functions. In addition, docetaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

On literature survey reveals that there is no sophisticated method for estimation of Docetaxel in tablet dosage form in common laboratories. So the present study related to the development of a new analytical method for estimation of Docetaxel in tablet dosage form. Validation of the proposed analytical method was done as per ICH guidelines.

The Chemical Formula for Docetaxel is $C_{43}H_{53}NO_{14.}$ The Molecular weight[6] of Docetaxel is 807.8792g/mol. The IUPAC Name of Docetaxel is (1S,2S,3R,4S,7R,9S,10S,12R,15S)-4-(acetyloxy)-15-{[(2R,3S)-3-{[(tert-butoxy)carbonyl]amino}-2-hydroxy-3phenylpropanoyl]oxy}-1,9,12-trihydroxy-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.0^{3},^{10}.0^{4},^{7}]heptadec-13-en-2-yl benzoate. The structure of Docetaxel is shown in fig-1.

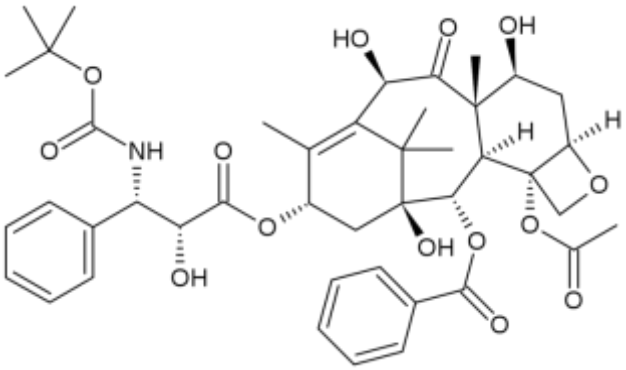

Fig-1: Structure of Docetaxel

II. METHODOLOGY

2.1 Materials

The Docetaxel working standard was received from Syncorp Clincare Technologies Pvt. Ltd, Hyderabad. Docetaxel drug substance from Cipla labs, Mumbai. Docetaxel Injection 20mg, Orthophosphoric acid, Potassium dihydrogen orthophosphate of grade LR. Acetonitrile, methanol of grade HPLC. HPLC Grade water, hydrochloric acid, sodium hydroxide, hydrogen peroxide of grade pure.

2.2. Chromatographic Conditions

The analysis was carried on HPLC Symmetry ODS C_{18}, 5µm, 25cmx4.6mm i.d. with detection[7] wavelength of 235nm. Injection volume of 20.0 µL and maintaining flow rate at 1mL/min.

2.3. Mobile Phase Preparation

The mobile phase used in this analysis consists of a mixture of Phosphate Buffer & pH[8] adjusted to 3.6 with orthophosphoric acid) and Acetonitrile in a ratio of 27:73.

270ml of this buffer solution was added and properly mixed with 730ml of Acetonitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 5 minutes before using in the experiment.

2.4. Diluent Preparation

Mobile phase[9] can be used as diluent.

2.5. Preparation of Standard Solution

Weigh accurately 25mg of Docetaxel standard[10] and transferred into clean and dry 25ml volumetric flask. Then finally make up to the mark with mobile phase.

Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.6. Preparation of Test Solution

Twenty Capsules were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25ml with same solvent. Then 10 ml of the above solution was diluted[11] to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five into the HPLC system.

III. Method Validation

3.1. Accuracy:

Recovery study:

To determine the accuracy[11] of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Docetaxel were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values[12] were calculated. The results were shown in table-1.

3.2. Precision:

3.2.1. Repeatability

The precision[13] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Docetaxel (API) the percent relative standard deviations[14] were calculated for Docetaxel is presented in the Table-2.

3.2.2. Intermediate Precision:

3.2.2.1. Intra-day & inter-day:

The intra & inter day[15,16] variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Docetaxel revealed that the proposed method is precise.[29]

3.3. Linearity & Range:

The calibration curve[17] showed good linearity in the range of 0-14µg/ml, for Docetaxel (API) with correlation coefficient (r^2) of 0.999 (Fig-4). A typical calibration curve has the regression equation[18] of y = 72353x + 5437. for Docetaxel.

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging[19] from 6, 8, 10, 12 and 14µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions[20]. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). The results which are given in Table below were within acceptable limits.

3.4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate[21] (±0.1ml/min), Temperature[22] (±2^{0}C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness[23,24] of the method are also in favour of (Table-5, %RSD< 2%) the developed RP-HPLC method for the analysis of Docetaxel (API).

3.5. LOD & LOQ:

Limit of Detection[25] (LOD) and Limit of Quantification[26] (LOQ) are terms used to describe the smallest concentration of analyte that can be reliably measured by an analytical procedure.

The LOQ may be equivalent to the LOD or it could be at a much higher concentration.

The LOD and LOQ were calculated by the use of the equations

$LOD = 3.3 \times \sigma / S$ and

$LOQ = 10 \times \sigma / S$

Where,

σ is the standard deviation of intercept of Calibration plot and

S is the average of the slope of the corresponding Calibration plot.

3.6. Assay of Docetaxel in dosage form:

DOCETAXEL 20mg

Twenty dosage forms were taken and the I.P. method was followed to determine the average weight. Above weighed tablets[27] were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of Hplc grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection[28] of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-6.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of Test obtained with test preparation

AS = Peak Area of Standard obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

IV. RESULTS AND DISCUSSION

4.1. Accuracy:

Table-1: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.157	595625	101.962	Mean= 101.387% S.D. = 0.516599 % R.S.D.= 0.509532
S_2 : 80 %	8	8.099	591457	101.237	
S_3 : 80 %	8	8.077	589875	100.962	
S_4 : 100 %	10	10.077	734587	100.77	Mean= 100.43% S.D. = 0.833727 % R.S.D.= 0.830157
S_5 : 100 %	10	9.948	725268	99.48	
S_6 : 100 %	10	10.104	736524	101.04	
S_7 : 120 %	12	11.989	872949	99.908	Mean= 100.6997% S.D. = 0.841254 % R.S.D.= 0.835409
S_8 : 120 %	12	12.190	887456	101.583	
S_9 : 120 %	12	12.073	878975	100.608	

4.2. Precision:

4.2.1. Repeatability:

Table-2: Repeatability Results of Docetaxel

HPLC Injection Replicates of Docetaxel	Retention Time	Peak Area
Replicate – 1	3.461	726541
Replicate – 2	3.461	724857
Replicate – 3	3.462	723541
Replicate – 4	3.461	725268
Replicate – 5	3.459	728984
Replicate -6	3.459	725745
Average	**3.4605**	**725822.7**
Standard Deviation	**0.001225**	**1841.86**
% RSD	**0.035392**	**0.253762**

4.2.2. Intermediate Precision:

4.2.2.1. Intra-day and Inter-day:

Table-3: Results of intra-assay & inter-assay

Conc. Of Docetaxel (API) (µg/ml)	Observed Conc. Of Docetaxel (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.02	1.05	7.96	1.06
10	10.10	0.96	10.06	0.99

12	11.89	0.85	12.03	0.92

4.3. Linearity and Range:

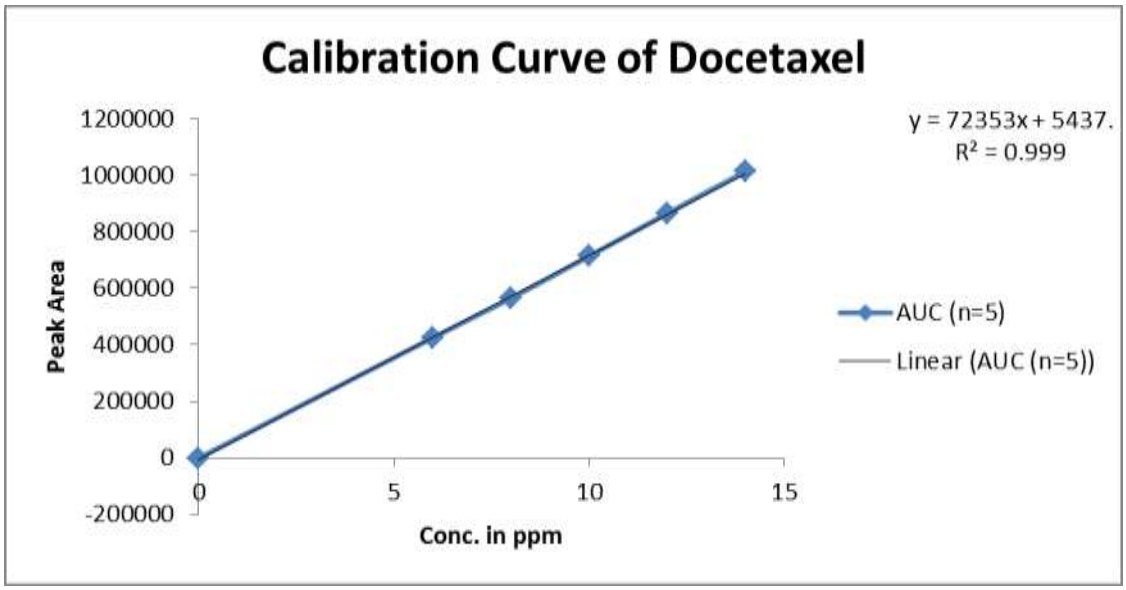

Fig-2: Calibration Curve of Docetaxel (API)

Table-4: Linearity Results of Docetaxel

CONC.	AUC (n=5)
0	0
6	425874
8	565872
10	714542
12	865632
14	1013121

4.4. Robustness:

Table-5: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.49
Flow (0.9 ml/min)	0.63
Temperature (27^0C)	0.54
Temperature (23^0C)	0.68
Wavelength of Detection (237 nm)	0.56
Wavelength of detection (233 nm)	0.84

4.5. LOD and LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.29 µg/ml respectively for Docetaxel which represents that sensitivity of the method is high.

4.6. Assay of Docetaxel in Pharmaceutical dosage form

Table-6: Assay of DOCETAXEL

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Docetax (Cipla Pharmaceuticals Limited)	20mg	19.52 (±0.06)	98.075 (±0.49)

5. CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Docetaxel API. Further the developed RP-HPLC method has excellent sensitivity, precision, accuracy and reproducibility.

The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Docetaxel in different formulations.

The Forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Docetaxel under various forced degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations.

Finally it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

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